AWARD NUMBER: W81XWH-15-1-0216

TITLE: Vascular and Skeletal Muscle Function in Gulf War Veterans Illness

PRINCIPAL INVESTIGATOR: Scott Kinlay, MBBS, PhD

CONTRACTING ORGANIZATION: Boston VA Research Institute, Inc.
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14. ABSTRACT

Gulf War Illness (GWI) is a constellation of symptoms including fatigue, musculoskeletal pain, memory loss, and mood changes reported by Gulf War Veterans shortly after their return in 1991. Approximately 40% of Gulf War Veterans (over ¼ million Veterans) have GWI by the Center for Disease Control criteria for GWI (a recommended method for defining GWI). The underlying causes of GWI are poorly understood. The overall goal of our study is to determine if there are differences in blood vessels, skeletal muscle performance, and their controlling proteins and genes in Gulf War Veterans with and without GWI. Abnormalities in these factors may explain the symptoms of fatigue and muscle pain that are major parts of GWI. These insights could lead to new treatments for GWI as well as other illnesses with similar symptoms. Our pilot data show that we can assess blood flow to muscle, muscle strength and fatigue and examine proteins and genes from a specimen of muscle in Gulf War Veterans. We will assess if abnormalities in these factors are potential explanations for GWI. This study is seeking to enroll 70 Veterans (35 with GWI and 35 without GWI) and is currently open to enrollment.

15. SUBJECT TERMS
Gulf War Syndrome, Persian Gulf Syndrome/physiopathology, Veterans

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1. **INTRODUCTION:** Gulf War Illness (GWI) is a constellation of symptoms including fatigue, musculoskeletal pain, and neurocognitive reported by Gulf War Veterans shortly after their return from deployment in 1991. The Center for Disease Control and Prevention (CDC)'s clinical diagnostic criteria for GWI is one of two recommended by an Expert Committee, and is based on symptoms in three categories: fatigue, mood/cognition, and musculoskeletal symptoms. Currently, approximately 40% of Gulf War Veterans (over ¼ million Veterans) have GWI by these criteria. The pathophysiological mechanisms underlying GWI are not understood and insights into these mechanisms could lead to new treatment interventions. Furthermore, abnormalities in peripheral blood flow related to endothelial function and muscle bioenergetics due to environmental toxins, such as those present in the Gulf War, are plausible mechanisms that could relate to the musculoskeletal symptoms of GWI. This study will determine the pathophysiology, and related genome and transcriptional mechanisms related to endothelial function and muscle mitochondrial biogenesis in Veterans with and without GWI through a case-control design of 70 Veterans who have severed in the Gulf War and are participants of the ongoing Fort Devens Cohort. Specific aims include comparisons of: (1) microvascular endothelium-dependent and endothelium-independent function of the profunda femoral artery using techniques commonly used for peripheral endovascular interventions, (2) peak oxygen uptake and ventilator anaerobic threshold during cardiopulmonary exercise testing and other muscle functions, (3) expression of genes relevant to endothelial function and mitochondrial function in muscle biopsy samples, and (4) gene polymorphisms related to endothelial and mitochondrial respiratory function.

2. **KEYWORDS:**
Gulf War Syndrome
Persian Gulf Syndrome/physiopathology
Veterans

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Major Tasks	**Timeline (months)**	**Status**
Major Task 1: Institutional Review Board (IRB) Approval		
Modify the current protocol to add new experiments and aim (microarray assays and next-generation RNA sequencing)	0.5	Completed
Submit final protocol to VA Boston Healthcare System (VABHS) IRB	0.5	Completed
Milestone: Achieve local IRB approval of protocol	1	Received VA Boston IRB approval for modified protocol amendment, Protocol Version 2.0 on 11-JAN-2016. Submitted to and received HRPO Initial Approval for protocol (version 2/ dated 23-Dec-2015) on 09-FEB-2016.

Major Task 2: Recruitment of Subjects		
Send batch invitations to 400 Gulf War Veterans who have completed the Fort Devens cohort study	1-24	Dr. Maxine Krengel (Co-Investigator) helped initiate recruitment by sending Gulf War Veterans invitation letters to participate around 19-FEB-2016. As of 30-JUN-2017, 895 Veterans have been letters of invitation at least once. Recruitment plans includes sending a minimum of 3 letters and if no responses are received from letters, telephone call will be made to inquire about interest.
Major Task 3: Endothelial Function Study and Muscle Biopsy		
Schedule Visit 1 (Endothelial function studies and muscle biopsies)	1-24	Appointments for Visit 1 continue to be scheduled for all interested Veterans.
Complete endothelial function studies and muscle biopsies and measurement of intravascular ultrasound and flow data to assess microvascular and conduit endothelial function	1-28	As of 30-JUN-2017, 49 Veterans have complete all procedures required of Visit 1 which includes successful complete of endothelial function study and muscle biopsy. Of 49 enrolled Veterans, intra-arterial flow data was unavailable for 7 Veterans, resulting in 42 Veterans with complete Visit 1 data from all required procedures.
Milestone: Complete endothelial function data and muscle biopsies on 70 subjects	28	Pending
Major Task 4: Exercise and Cardiopulmonary Stress Testing		
Schedule Visit 2 (Exercise and cardiopulmonary stress tests)	3-28	Scheduling for Visit 1 continues to occur at least 2 weeks after completion of Visit 1, limited by Veteran's availability.
Complete exercise and cardiopulmonary stress studies and interpretation	3-28	As of 30-JUN-2017, 45 Veterans have successfully completed Visit 2.
Milestone: Complete exercise data on 70 subjects	28	Pending

Major Task 5: Histopathology and Electron Microscopy		
Prepare muscle biopsy specimens for histopathology and electron microscopy and image	4-30	Pending
Complete data on muscle analysis including histopathology	4-30	Pending
Milestone: Complete histopathological data and electron microscopy data on representative subjects	30	Pending
Major Task 6: Gene and protein expression relating to mitochondrial biogenesis		
Isolate DNA, RNA, and protein from muscle tissue samples. Prepare cDNA from RNA samples.	4-28	Pending
Complete qPCR and Western Blot to assess genes and proteins regulating mitochondrial biogenesis.	4-30	Pending
Milestone: Complete data on specific genes and proteins regulating mitochondrial biogenesis on 70 subjects	30	Pending
Major Task 7: Transcriptome microarrays comparing cases and controls		
Run microarrays at Dana Farber Microarray Core Lab from cDNA samples	24-30	Pending
Interpret results and identify candidate genes related to Gulf War Illness	24-30	Pending
Milestone: Complete analysis of transcriptome microarray data on 70 subjects	30	Pending
Major Task 8: SNP Microarray		
Run MVP microarray at Dana Farber Microarray Core Lab	4-28	Pending
Identify candidate genetic polymorphisms related to GWI	4-30	Pending
Milestone: Complete data analysis of SNP microarray data on 70 subjects	30	Pending
Major Task 9: Finalize data analysis, present results and meetings, publish results		
Complete statistical analyses including comparisons of cases and controls and prepare for publication, presentation, and public release of de-identified data for other researchers.	24-36	Pending

What was accomplished under these goals? This report summarizes the research progress in the most recently completed budget period from July 1, 2016 to June 30, 2017. This time period corresponds to the second of this three-year project. The objectives of this study is to investigate the hypothesis that when compared to Veterans without Gulf War Illness (GWI), Veterans with GWI will have differences in arterial endothelial function, muscle function determined by cardiopulmonary exercise testing, and expression of genes responsible for mitochondrial function. This is a case control study of 2 visits looking to enroll 70 participants (35 with GWI and 35 without GWI) from a well characterized cohort of Gulf War Veterans (the Fort Devens study). Study Visit 1 consists of an endothelial function test performed using standard cardiac catheterization techniques used for peripheral artery interventions, and a muscle biopsy of the vastus lateralis muscle. Study Visit 2 consists of cardiopulmonary exercise testing and other tests of muscle strength and endurance.

The second budget period was heavily focused on participant recruitment (Major Task 2, 3, and 4). In the last year, 49 (70%; of expected 70) Veterans have been scheduled for Study Visit 1. New letters of invitation continue to be mailed every 2-3 weeks and response continues to be strong.

What opportunities for training and professional development has the project provided? Nothing to Report.

How were the results disseminated to communities of interest? Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals? In the next reporting period, we are planning to meet subject recruitment goals and will continue to recruit Veterans of the Fort Devens cohort through invitation letters. The Fort Devens cohort consists of over 1300 Veterans. Veterans who do not respond to the initial letter will be mailed up to 2 reminder letters. Study Visit 1 and 2 will be scheduled and completed for all Veterans expressing interest in study participation.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project? Fatigue and musculoskeletal symptoms are major components of GWI and could have an important impact on other symptoms associated with GWI. There are plausible reasons why endothelial function and mitochondrial biogenesis in muscle may be affected by exposure to environmental toxins during the Gulf War and lead to these symptoms.

In particular, pyridostigmine and nerve gases are anticholinesterase agents that potentially have long term effects on the balance of cholinesterases and acetylcholine, which could affect activity at the neuromuscular junction of skeletal muscle, muscarinic receptors affecting vascular smooth muscle tone, and damage mitochondrial structure and electron transport activity in several tissues including muscle.

Insights on the pathogenesis of GWI could lead to new treatments for GWI, but also provide novel mechanistic insights into other exposure-related occupational health illness, such as pesticide exposure in the agricultural industry. Our study may also elucidate mechanisms of interest that require investigation as causes of other illnesses with muscle fatigue, pain, and abnormal muscle metabolism, such as peripheral artery disease and chronic heart failure, and advance our understanding of the pathophysiology of GWI and discover molecular pathways that could elucidate novel treatments for GWI. It may also direct future research into abnormalities of important molecules that could form the basis of an improved diagnostic test, although establishing a diagnostic test is not the focus of this proposal.

As the study is in the recruitment phase, there are currently no findings to report.

What was the impact on other disciplines? Nothing to Report.

What was the impact on technology transfer? Nothing to Report.

What was the impact on society beyond science and technology? Nothing to Report.

5. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change: Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them: As we are close to the recruitment target of 70 total Veteran participants, interim review of collected was performed in this last quarter. Of the 59 Veterans who have expressed interest in study participation, 49 have provided consent as of 30-JUN-2017. Of 49, 7 have incomplete Visit 1 data, resulting in 42 evaluable subjects. For the purposes of this study, an evaluable subject is one who has successfully completed both Study Visit 1 and Study Visit 2 and has analyzable data to include (but not limited to) intraarterial IVUS, intraaterial flow, cardiopulmonary test, and bone density scan. As successful endpoint evaluation is dependent on the data from 70 evaluable subjects, we are requesting approval from the VA Boston Institutional Review Board to increase total enrollment from 70 participants to approximately 80-100 participants. As of 30-JUN-2017, this amendment request has been submitted to the VA Boston Institutional Review Board and a response is expected in the next quarter.

Changes that had a significant impact on expenditures: Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: Nothing to Report.

6. **PRODUCTS:** Nothing to Report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

What individuals have worked on the project? Individuals who have worked on this project during the most recent budget period are described below with their efforts and contribution divided by each quarterly reporting period.

July 1, 2016 to September 30, 2016

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	1
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1.

Name:	Jacquelyn-My Do, MPH
Project Role:	Assistant Program Manager
Research Identifier:	N/A
Nearest person month worked:	3
Contribution to Project:	Ms. Do is the new Project Manager for this study and has assumed the administrative project management responsibilities of Dr. Sara Jones. She is planning and tracking study recruitment, maintaining regulatory approval, and managing project resources and budget. She oversees mailing of recruitment letter, scheduling of patient appointments, and manages data collection.

Name:	Margot Quinn, BA
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Quinn performs research activities are described in the study protocol, including informed consent of participants and conduct of Visit 1. She also assisted the Project Manager in administrative duties.

October 1, 2016 to December 31, 2016

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	1
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1.

Name:	Jacquelyn-My Do, MPH
Project Role:	Assistant Program Manager
Research Identifier:	N/A
Nearest person month worked:	3
Contribution to Project:	Ms. Do continues in her role as Project Manager for this study. She continues to track study progress, maintains regulatory approval, and manages project resources and budget. She also oversees mailing of recruitment letter, scheduling of patient appointments, and manages data collection.

Name:	Margot Quinn, BA
Project Role:	Research Assistant

Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Quinn performs research activities are described in the study protocol, including informed consent of participants and conduct of Visit 1. She also assisted the Project Manager in administrative duties.

January 1, 2017 – March 31, 2017

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	2
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1. Additionally, he is actively recruiting for a Project Manager to replace Ms. Do.

Name:	Jacquelyn-My Do, MPH
Project Role:	Assistant Program Manager
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Do continues in her role as Project Manager for this study until 20-JAN-2017. She continues to track study progress, maintains regulatory approval, and manages project resources and budget. She also oversees mailing of recruitment letter, scheduling of patient appointments, and manages data collection. As of 20-JAN-2017, she has left the Boston VA Research Institute and VA Boston. She continues a temporary assignment with the project to help gather and analyze data from Visit 1, slated to end at 30-APR-2017.

Name:	Margot Quinn, BA
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Quinn performs research activities are described in the study protocol, including informed consent of participants and conduct of Visit 1. She also assisted the Project Manager in administrative duties.

April 1, 2017 – June 30, 2017 (most recent)

Name:	Scott Kinlay, MBBS, PhD

Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	1
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1. He continues to actively recruit for a Project Manager to replace Ms. Do, but has hired a new research assistant to replace Margot Quinn.

Name:	Margot Quinn, BA
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Quinn continues to performs research activities as described by study protocol, including informed consent of participants and conduct of Visit 1. As of 30-JUN-2017, she will be leaving her appointment with BVARI and will not longer be working on this project.

Name:	Melissa Chin, BS
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Chin has a Bachelor of Science in Biochemistry from Boston College and has extensive bench lab experience. She started on this project on 26-JUN-2017 and will be replacing Ms. Quinn's position as research assistant, responsible for recruitment and activities of study visit 1.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? Nothing to Report.

What other organizations were involved as partners? When subject recruitment is complete, we will begin sample analysis. This will include isolation and analysis of RNA and DNA, protein isolation expression, microchip arrays, and next generation RNA generation. We plan to work with our collaborator, Dr. Calum MacRae at the Brigham and Women's hospital to complete these analyses.

Organization Name: Brigham and Women's Hospital
Location of Organization: Boston, MA
Partner's contribution to the project: Collaboration

Until sample analysis commences, we current do not have any partner organizations.

8. **SPECIAL REPORTING REQUIREMENTS COLLABORATIVE AWARDS:** None.

9. **APPENDICES:** Please see the attached quad chart.

Vascular and Skeletal Muscle Function in Gulf War Veterans Illness

Log Number: GW14003

Award Number: W81XWH-15-1-0216

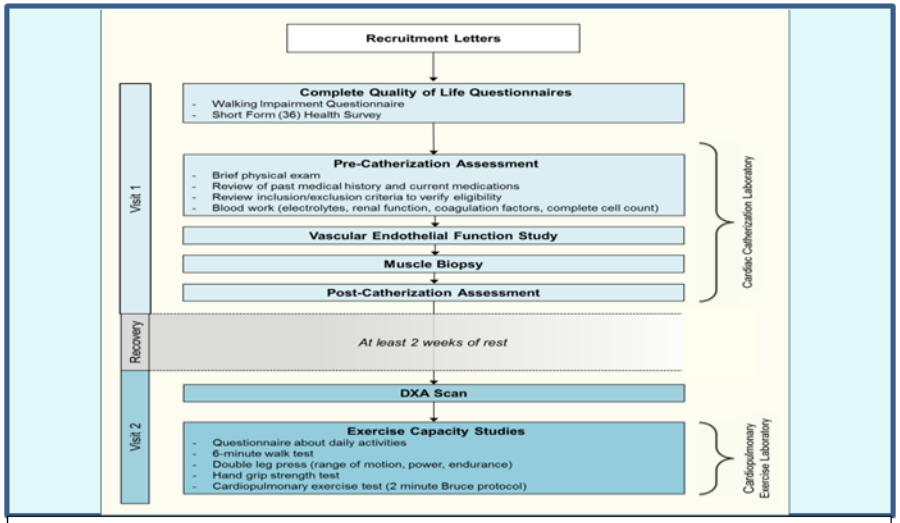
PI: Scott Kinlay, MBBS, PhD **Org:** Boston VA Research Institute, Inc. (BVARI) **Award Amount:** $870,642.00

Study/Product Aim(s)

- To determine if microvascular endothelium-dependent and endothelium-independent function of the profunda femoral artery is impaired in subjects with Gulf War Veterans Illness (GWVI) compared to deployed Veterans without GWVI.
- To determine if peak oxygen uptake and ventilatory anaerobic threshold during cardiopulmonary exercise testing, and other muscle functions are impaired in subjects with GWVI compared to deployed Veterans without GWVI.
- To determine how the expression of genes relevant to endothelial function and mitochondrial function in muscle biopsy samples differs between subjects with GWVI compared to deployed Veterans without GWVI.
- To determine if polymorphisms to genes relating to endothelial function and mitochondrial respiratory function differ between subjects with GWVI compared to deployed Veterans without GWVI.

Approach

Gulf War Illness (GWI) is a constellation of symptoms including fatigue, musculoskeletal pain, and neurocognitive dysfunction reported by Gulf War Veterans shortly after their return from deployment in 1991. There are plausible reasons why endothelial function and mitochondrial biogenesis in muscle may be affected by exposure to environmental toxins during the Gulf War and lead to GWI symptoms. We hypothesize that compared to Veterans without GWI, Veterans with GWI will have differences in arterial endothelial function, muscle function determined by cardiopulmonary exercise testing, and the expression of genes responsible of mitochondrial function.



Accomplishment: This IRB-approved prospective cross-sectional clinical trial will consist of 2 study visits. 70 Gulf War Veterans (35 with GWI and 35 without GWI) will be enrolled.

Timeline and Cost

Activities / CY	15	16	17	18	19
Milestone 1: Achieve local IRB approval of protocol					
Milestone 2: Complete Visit 1 (endothelial function and muscle biopsies) on 70 subjects					
Milestone 3: Complete Visit 2 (exercise and cardiopulmonary stress test) onn70 subjects					
Milestone 4: Complete histopathology and electron microscopy analysis					
Milestone 5: Complete gene and protein analysis					
Milestone 6: Complete analysis on transcriptome microarray data					
Milestone 7: Complete analysis of SNP microarray data					
Finalize data anlysis, present results and meetings, publish results					
Estimated Budget ($K)	**$0**	**$220**	**$361**	**$287**	**$0**

Updated: 30-JUN-2017

Goals/Milestones

CY15 Goals – Institutional Review Board (IRB)

☑ Achieve local IRB approval

☑ Achieve HRPO approval

CY16/17 Goals – Subject Recruitment

☑ Start recruitment with letters of invitations

☑ Schedule and conduct Visits 1 and 2

CY18 Goals – Complete recruitment and data analysis

☐ Complete Visits 1 and 2 on 70 Subjects

☐ Complete histopathological data, electronic microscopy data, specific genes and proteins regulating mitochondrial biogenesis, analysis of transcriptome microarray data on samples collected

CY19 Goal – Analyze and publish results

☐ Analyze, present, and publish results at DoD and scientific meetings

Comments/Challenges/Issues/Concerns: We anticipate that a majority of the expenditures will be used to cover costs of analysis.

Budget Expenditure to Date

Projected Expenditure: $361,000; Actual Expenditure: $118,429